THE REACTION AND PHYSIOLOGY OF THE HEPATIC DUCT AND CYSTIC BILE OF VARIOUS LABORATORY ANIMALS

EXPERIMENTAL TYPHOID-PARATYPHOID CARRIERS. VI

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In the preceding papers we have shown that typhoid bacilli are frequently discharged in the bile of practically every animal that has been inoculated intravenously with large doses of bacteria of the typhoid-paratyphoid group. Accepting the statements and interpretations of others, it became quite evident that the biliary secretions were under suspicion as important factors in the development of carriers.

Since Nichols issued his studies on the supposedly antiseptic effect of rabbit bile, we were compelled to verify the published facts and to correlate the findings of others with our own. In this connection we studied primarily the antiseptic action of animal and human bile. As Nichols [1] considered the latter to be largely due to its alkalinity, we measured the H-ion concentration of biles derived from a large number of rabbits, guinea-pigs, dogs, rats, cats, etc. We found rather constant difference in the reaction between the bile derived from the liver by a common duct fistula and the one obtained from the gallbladder. We noted that the former became more alkaline on standing outside of the viscus, a final H-ion concentration above P_H 9.0 being frequently reached. Most of our determinations were made on animals that had been kept on controlled diets, and the bile specimens were obtained by temporary common ducts fistulas. In the course of the collection of these samples a number of physiologic data which are rarely mentioned in the literature were recorded. They contribute important information to the physiology of biliary secretions and to the function of the gallbladder, and are therefore included in this paper.

No exact data as to the H-ion concentration of the hepatic duct and gallbladder bile of rabbits, guinea-pigs, rats, monkeys and man were available. Preliminary tests were made by the titration method, but we were convinced that the titrable acidity or alkalinity did not repre-

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[1] J. Exper. Med., 1916, 24, p. 497.

sent the true H-ion concentration of the bile specimen. In measuring the H-ion concentration it was also recognized that special precaution had to be observed in order that the escape of CO_2 did not decrease measurably the H-ion exponent. As stated in the introduction, we have also compared, where possible, the reaction and physiology of gall-bladder bile with the hepatic duct bile. In a limited number of experiments we tested the influence of diet on biles, the biles from carrier animals, and followed the changes of bile samples, which had been neutralized in vitro.

METHODS

The hepatic duct bile samples were collected from temporary common duct fistulas. The technical procedures employed have been detailed in the preceding paper.

Cystic bile was procured at necropsies or by laparotomies, a capillary Pasteur pipet being inserted through the seared gallbladder wall and the contents aspirated. In normal healthy animals invariably sterile bile was present. Our observations fail to support the claims of Ehret and Stolz,[2] who recorded that 11 of 17 guinea-pigs' bile tested were contaminated with various organisms. Gallbladder bile of large, domesticated animals was obtained from the slaughter house. The isolated cystic duct was tied and the whole bladder wrapped in gauze soaked with bichloride solution. In the laboratory the bile was aspirated by means of a Pasteur pipet, which was introduced through a cauterized area of the bladder wall. In contrast to Ehret and Stolz, Létienne [3] and others, the samples obtained in this manner were sterile in 90% of the instances.

In studying the reaction of the bile specimens we had access to two methods: the titration method and the colorimetric method. The titration method was handled by us thus: One part of bile diluted in 9 parts of freshly boiled and cooled neutral distilled water mixed with phenolphthalein (0.1 c c of a 2% solution in 50% alcohol to 10 c c of diluted bile solution) was titrated with the aid of a micro-burette (0.1 c c pipet) with a decinormal solution of a sodium hydroxide or hydrochloric acid as the case may be. The end-point arbitrarily chosen by us was the first perceptible change in color recorded during the first minute. The solutions of hepatic duct bile were always clear, and a slight change was readily noted in contrast with the control. Heavily pigmented or turbid

[2] Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1900, 6, p. 350; 7, p. 372.
[3] Arch. de méd. expér. et d'anat. path., 1891, 1, p. 761.

cystic biles of man, monkey, dog, etc., were more difficult to titrate. The first discernible change in color covers a broader range and consequently may easily increase the experimental error. Individual differences of interpretation were eliminated by the same person always making the readings.

We have deviated from the methods of Jolles [4] and others in slightly varying the amount of indicator used rather than the diluent. For example, we have uniformly used 0.1 c c of phenolphthalein solution for amounts of 0.1 c c to 1 c c of bile. And again in dark and turbid biles we have found it simpler and more expeditious to double or even to quadruple the quantity of indicator rather than to increase the amount of diluent. The comparatively small amounts of bile aspirated from the gallbladder of guinea-pigs and rabbits that was to be titrated before cultivating forced us to titrate only 0.1 c c lots of bile, in which instances the reaction was determined with N/50 solutions of alkali and acid. For measuring the alkalinity of bile samples sensitive azolitmin indicator solution [5] was used in the same manner. However, for the sake of economy in titrations an approximate range of the reaction of biles was first obtained by indicator papers or solutions.

For reasons to be discussed, the colorimetric method for the H-ion determination was adapted for the study of the reaction of bile samples. It is generally accepted that only the accurate determination of the actual hydroxyl-ions should be used to express the reaction of mediums or biologic fluids. Before the publication of Clark and Lubs [6] dealing with a dependable procedure had appeared, and a set of brilliant indicators had been found, we followed the outline described by Hurwitz, Meyer and Ostenberg.[7] It is quite natural that the method of Clark and Lubs, which covers a broader range of reaction, should have replaced the obsolete procedure adopted at the beginning. This in part will explain why some of our results dealing with P_{H^+} determinations are expressed as >8.6 and >9.2.

The reaction of the bile is progressively changing and therefore the electrometric method, being time-consuming, would favor erroneous results. However, for old biles exposed to various factors for several days, the electrometric method should be used more frequently. Unfortunately, we were not in possession of an equipment to make such read-

[4] Arch. f. d. ges. Physiol., 1894, 67, p. 1.
[5] Sörensen: Ztschr. f. phys. Chemie, 1909, 64, p. 120.
[6] J. Bacteriol., 1917, 2, pp. 1, 109 and 191.
[7] Johns Hopkins Hosp. Bull., 1917, 2, p. 2.

ings when necessary, but through the courtesy of Dr. Schmidt, several samples were checked electrometrically, and the P_H^+ readings were found to correspond closely with those obtained by the colorimetric method. For biologic work, minor differences of one or two degrees are of considerably less importance than in accurate chemical work. We have recently tested simultaneously a number of old hepatic duct bile samples of the rabbit by the electrometric and colorimetric method and have convinced ourselves that the readings made by the latter are absolutely reliable.

As already suggested in the introduction, it was our purpose to determine the reaction of biles, hepatic duct or cystic, in vitro with the least exposure to disturbing factors. It is therefore a great advantage to note from the paper of Clark and Lubs [8] that the "dilution method" is with safety applicable to the H-ion determination of turbid fluids. They established the legitimate dilution as 1:5, but they also mentioned that highly colored solutions may be diluted to a point where they may be used in the comparator. Furthermore, they showed [9] that the 1:10 dilution is reliable for approximate determinations upon a wide variety of solutions highly colored and turbid (p. 193). We have found that a dilution of 1:10 influences the final P_H^+ readings slightly. It is possible to check this conclusion on lightly colored biles (hepatic duct of guinea-pigs, dogs, etc.) in an undiluted state. A sufficiently large series of comparative tests with highly colored biles has convinced us that a dilution of 1:10 shows approximately the same P_H^+ values as the one of 1:5 and has also been proved by electrometric determinations.

It has been emphasized that small amounts of bile are in this study the only available material. The dilution method is the only feasible procedure by which we were able to obtain comparable data on the behavior of hepatic duct and cystic bile of one and the same animal. Therefore we have adopted for colorimetric determinations of all biles a 1:10 dilution (0.2 c c bile in 1.8 c c freshly boiled and cooled distilled water mixed with 0.2 c c of indicator). Invariably the P_H^+ determinations were made with two or more indicators, which cover our ranges, namely: brom thymol blue, phenol red, cresol red and thymol blue. It is always advisable to use at least two indicators overlapping the probable range of the H-ion concentration of the bile. Shades of color which develop under varying CO_2 tensions and which make comparison

[8] Footnote 6, p. 129.
[9] Footnoe 6, p. 191.

TABLE 1

TITRABLE ACIDITY AND ALKALINITY OF VARVIOUS BILE SAMPLES

Animal	Diet	Method of Obtaining Bile	Gallbladder Bile					Hepatic Duct Bile				
			Number of Biles Tested	Titrable Acidity Phenolphthalein	Degree of Acidity	Titrable Alkalinity Phenolphthalein	Degree of Alkalinity	Number of Biles Tested	Titrable Acidity	Degree of Acidity	Titrable Alkalinity	Degree of Alkalinity
Rabbit	Mixed	Chloroformed	15	0.8 (0.1-2.7)	0.032	0	0	7	0.13 (4)* (0.1-0.2)	0.52	0.18 (5) (0.1-0.2)	0.064
	Immunized	Or laparotomy						7	0.14 (5) (0.01-0.2)	.....	0.18 (3) (0.1-0.3)	0.064
	Immunized	After death	6	3.5 (2.0-6.4)	1.40	0	0	....	.....	.....	.....	.....
	Immunized carriers	Laparotomy	18	0.2 (3) (0.1-0.4)	0.08	0.39 (amphotheric —1.1) (15)	0.1404	....	.....	.....	.....	.....
	Immunized carriers	After death	3	1.5 (0.5-4.0)	0.60	0	0	....	.....	.....	.....	.....
	Oats	Laparotomy	3	0.46 (0.4-0.6)	0.184	0	0	7	0.06 (2)	0.024	0.15 (5)	0.054
	Wheat	Laparotomy	2	1.12	0.448	0	0	2	0.06 (2)	0.024	0.05 (1)	0.018
	Cabbage	Laparotomy	6	0.61	0.244	0	0	9	0.01 (5)	0.004	0.29 (7)	0.104
	Carrots	Laparotomy	2	0.65	0.260	0	0	2	Amphotheric (1)	.....	0.05 (1)	0.018
	Cholestearolemia	Laparotomy	1	0.2	0.08	0	0	3	0	0	0.13 (3)	0.046
	Alkalosis	Laparotomy										
	Sodium bicarbonate	Laparotomy	3	0.57	0.228	0	0	4	0.3 (1)	0.12	0.16 (3)	0.057
	Acidosis with HCl	Laparotomy	3	0.77 (0.1-2.5)	0.308	0	0	4	0.27 (3)	0.18	0.2 (1)	0.072
	Starvation	Laparotomy	2	1.32	0.528	0	0	2	0.15 (1)	0.06	0.2 (1)	0.072
Guinea-pigs	Mixed	Laparotomy	47	0	0	0.43 (0.1-1.8)	0.1548	6	0	0	0.55 (6)	0.198
Rats	Mixed	Laparotomy	....	.....	.....	.....	.....	2	0.1 (1)	0.04	0.3 (1)	0.108
Dog	Mixed	Laparotomy and after death	33	1.15	0.460	.....	.....	5	0.15-0.2	0.06	.....	.....
Cat	Mixed	Laparotomy	2	1.6	0.64	.....	.....	2	0.2	0.08	.....	.....
Monkey	.....	Laparotomy and after death	7	1.6	0.64	.....	.....	1	0.75-1.0	0.3	.....	.....
Oxen	December	2 hours after slaughtering	8	0.55	0.220	.....	.....	....	.....	.....	.....	.....
Sheep	December	2 hours after slaughtering	9	1.9	0.76	.....	.....	....	.....	.....	.....	.....
Goats	December	2 hours after slaughtering	2	0.75	0.30	.....	....	1	0.35	0.14	.....	.....
Pigs	December	2 hours after slaughtering	10	1.05	0.42	.....	.....	....	.....	.....	.....	.....
Man	Biliary fistula	Immediately	....	.....	.....	.....	.....	1	0.1-0.2	0.04-0.08	.....	.....
	Sodium bicarbonate and Carlsbader salt	.....	....	.....	.....	.....	.....	....	2.2 to lacmoid	.....	.....	.....
	Light diet	Cholecystectomy	2	0.8 (1.0-0.6)	0.32	.....	.....	....	.....	.....	.....	.....

* Numbers in brackets refer to the number of animals tested.

difficult with one indicator, frequently can be matched by another, for example, thymol blue is superior to cresol red.

Pyrex glassware was used in all titration procedures. At the beginning of our studies the bile specimens were collected in open 15 c c graduated centrifuge tubes and when filled a colorimetric determination was immediately made. In order to avoid exposure of the bile to the factors changing the reaction, we resorted to the aspiration of the flowing hepatic duct bile by means of a hypodermic "Record" or any tightly fitting syringe. In the course of our inquiry, we finally developed a procedure that accomplishes a similar result in a more convenient manner, namely, the bile is collected directly under a 2 to 3 cm. layer of paraffin oil.

Cystic biles were removed by means of a hypodermic syringe or a Pasteur pipet and transferred to stoppered vials. They were either immediately titrated and tested or placed under oil for a short time.

To avoid a misunderstanding or repetition, we desire to specify that bile specimens standing in test tubes covered with or without oil for from 6 to 10 hours at room temperature or in the ice-chest and stoppered loosely by cotton or cork are considered "old biles."

RESULTS WITH RABBIT BILE

We examined 64 gallbladder and 55 hepatic duct biles of rabbits by the titration and by the colorimetric method. More than 100 gallbladder biles were tested by the colorimetric method only. The results dealing with the titrable acidity and alkalinity are shown in table 1. With the exception of the samples of cystic bile derived from "carriers," which will be discussed separately, all samples were definitely acid to phenolphthalein. The degree of acidity varied from 0.032 to 1.40 mg. of NaOH necessary to neutralize 1 gm. of bile. Rabbits fed on a mixed hay, oats and cabbage diet showed gallbladder bile of lowest acidity; those kept on wheat or starved, or fed with HCl, produced cystic biles that were distinctly acid. The most pronounced acid reaction was noted for gallbladder biles of rabbits which were not necropsied for at least from 5 to 10 hours.

The reaction of the hepatic duct bile of the same rabbits was either amphoteric, very slightly acid, or of various gradations from slightly to decidedly alkaline. About one-half of the specimens immediately titrated were alkaline to phenolphthalein, in contrast to the constant acid reaction of the gallbladder biles. The base-forming diets, particularly cabbage, produced hepatic duct biles, which were distinctly alkaline to phenolphthalein; 1 gm. of bile required 0.104 mg. of HCl for neutrali-

zation, using azolitmin as an indicator. Intravenous injections of $NaCO_3$, or feeding of the same substance in combination with base forming diet, gave common duct bile samples which differed little from those obtained from rabbits kept on mixed feeds.

The phenomenon of gradual increase of the alkalinity on standing was also noted when using the titration method. For example, a bile specimen giving a titrable acidity of +0.25 may on standing become amphoteric to phenolphthalein in 24 hours. It is evident that this reaction of the hepatic duct bile is different from that of the gallbladder. As a rule the titrable reaction of the former is more variable and more inclined to the acid side. All hepatic bile specimens were distinctly alkaline to litmus, 23 (or 58%) of 40 gallbladder biles tinged red litmus paper blue, 7 (or 17.7%) were amphoteric and 10 (or 25%) merely changed it to yellow.

TABLE 2

H-ION CONCENTRATION OF GALLBLADDER BILE OF ANIMALS FED ON VARIOUS DIETS

	Number of Animals	P_H+	Average P_H+
Mixed diet	15	6.6-7.2	6.7
Mixed diet and fasting	10	5.8-6.8	6.4
Acid forming diet (oats)	5	6.4-7.4	7.0
Experimental acidosis	3	6.4-6.8	6.6
Base forming diets	10	7.0-8.2	7.7
Experimental alkalosis	3	6.8-8.4	7.6

These differences became more apparent when we determined the H-ion concentration of the individual bile samples. Immedately after withdrawal from the gallbladder the bile showed an H-ion concentration expressed in a P_H+ ranging from 5.7-7.4. The average reaction varied between P_H+ 6.7-7.0, that is to say, the cystic bile of the rabbit was either acid or neutral or slightly alkaline. It may, however, become strongly alkaline on standing or give an initial P_H+ reading above 7.2 when the secretion has reached the gallbladder shortly before its removal.[10] Only in exceptional instances did we notice after 24 hours' standing a reaction of less than P_H+ 8.6. The factors responsible for this alteration will be analyzed. This variability of the reaction of the gallbladder bile is apparently existent in different individuals, and was in our experience closely connected with the general health and the diet of the animals. The figures in table 2 will illustrate this statement.

[10] The P_H+ readings of such specimens are recorded in the tables in brackets.

We were particularly interested in the determination of the factors influencing the H-ion concentration of the gallbladder bile and conducted a fairly large series of determinations. The figures noted must be considered strictly as averages; it is difficult to state with absolute certainty the origin of the fluid withdrawn from this organ. In animals with fistula it is not unlikely that hepatic duct bile has regurgitated into the bladder shortly before the sample has been removed and our determinations are made on liver instead of on bladder bile. For practical purposes, it is clear that mixed and acid-forming diets, including HCl are conducive to gallbladder biles which are slightly acid. This likewise is the case in fasting animals, in which there is apt to be stasis in the gallbladder. The reaction on standing remains either stationary or increases slowly. On the other hand, base-forming diets, such as cabbage and carrots and sodium bicarbonate feeding, produce alkaline cystic bile samples, which show a rapidly decreasing H-ion concentration on standing.

Hepatic duct bile, which is withdrawn from the collecting rubber tubing and immediately titrated, is always alkaline. Even when exposure to the air is reduced to a minimum, the P_H^+ reading never goes below P_H^+ 7.5, and in the majority of instances it is between P_H^+ 8.2-8.8. It will be noted from table 2 that the change in H-ion concentration is very rapid and that a bile sample examined 2 hours or more after removal from the body may give a P_H^+ reading from 8.4 to >8.6. This change is absolutely constant and is not markedly influenced by the general health of the animal or its diet. We shall discuss our experiments, which were conducted in an endeavor to produce such differences, after we have considered the factors responsible for the progressive changes on standing.

MECHANISM OF THE CHANGES IN H-ION CONCENTRATION OCCURRING IN FISTULA BILE ON STANDING

That changes occur in the reaction of biologic fluids on standing is well known, but to our knowledge particular attention has not been called to this phenomenon in connection with the titration of bile samples. Recent controversies dealing with the H-ion concentration of cerebrospinal fluids, and the careful studies of Levinson [11] suggested that the changes occurring in the hepatic bile on standing resulted from similar causes. A priori we could readily eliminate the conception that the

[11] J. Infect. Dis., 1917, 21, p. 556; Cerebrospinal Fluid, 1919, p. 96.

change was due to alkalies given off by glassware. Only carefully tested nonsol or pyrex test tubes were employed in our colorimetric determination. In analogy with the findings of other workers on biologic fluids, it suggested itself that the changes taking place on standing were either the result of loss of CO_2 or the formation or absorption of alkaline substances like ammonia.

We tested these conceptions by the following experiments: hepatic duct bile collected by aspiration or under paraffin oil in a test tube was divided into 6 portions of from 5 to 10 c c. The reaction of these samples at the beginning of the experiments was P_{H^+} 7.8-8.0. Sample 1 was covered with oil and tightly sealed with a rubber stopper; sample 2 was similarly closed, but without oil. Samples 3 and 4 were stoppered, leaving a small air space between the bile and the cork or rubber stopper. Sample 5 was placed in a test tube loosely closed by a cotton plug. Sample 6 was exposed in an open test tube in a desiccator to CO_2 and ammonia-free air. All samples were tested after 10 minutes, 1, 2 and 12 hours. The results of some of these experiments, which were repeated several times, are graphically summarized in chart I.

It may be concluded from these experiments (chart 1) that the H-ion concentration of hepatic duct bile decreases rapidly when the specimens are exposed to alkalies or to open air. The escape of CO_2 can be prevented or reduced by stratification with paraffin oil and tight sealing of the test tube with a cork or rubber stopper. The latter experiment also demonstrates that the decrease in the H-ion concentration is not the result of ammonia production in the fluid. In tubes tightly corked with air bubbles excluded, the bile either retained its original P_{H^+} for 12 to 24 hours or was only slightly above it (curves 1 and 2). On the other hand, exposure to NaOH or vacuum produced a rapid decrease of the H-ion concentration showing that accelerated loss of CO_2 is responsible for the increase of the P_{H^+}.

In a parallel set of experiments, hepatic duct bile collected in an open tube was distributed in lots of 5 c c each in test tubes, which were loosely plugged with cotton. The beginning H-ion concentration of the bile was P_{H^+} 8.2 (+0.35). Sample 1 was heated in an open water bath at 56 C. for 30 minutes in order to dispel quickly the CO_2; samples 2 and 3, both not heated, one stratified with paraffin oil and the other exposed to air, were placed with sample 1 in an open water bath at 37 C. Sample 4 was exposed in a chamber with free ammonia; sample 5 in an ammonia-free NaOH-desiccator, and sample 6 in a tube in which the air had been displaced by oxygen. These specimens were incubated at 37 C. P_{H^+} determinations were made on all specimens at 1, 2, 24 and 48-hour intervals (see chart 6, second paper).

It was noted that there is little difference in the H-ion concentration of hepatic duct bile when heated at 56 C. for 30 minutes or exposed to

air or to oxygen or to an ammonia-free atmosphere in a closed desiccator. However, the specimen exposed to ammonia vapors shows that bile is capable of absorbing it readily. The H-ion concentration was materially lowered. It is suggestive that while the bile may not readily form ammonia-like substances on standing, it may absorb substances like ammonia from the environment. This may in a measure explain the variability of the reaction of certain bile specimens when exposed to air or kept in a desiccator. It was frequently noted that one and the same specimen may show a low H-ion concentration (lowest electrometric reading on the 15th day P_{H^+} 9.214) when exposed to air, while the reaction of the secretion kept in a desiccator was not lowered in the same degree. In the first few days the desiccator tubes may, as a rule, give a slightly higher P_{H^+} reading than the air tube. However, this observation was frequently reversed and the air tube registered a lower H-ion concentration than the desiccator tube. We are under the impression that aside from the escape of the CO_2 another factor may influence the H-ion concentration. The fluctuating ammonia content of the laboratory atmosphere and the varying absorbability of this chemical by the bile may be responsible for the irregular P_{H^+} readings. Stratification with oil prevents the absorption of ammonia, but does not entirely inhibit the gradual escape of CO_2. Moreover the P_{H^+} increase cannot be the result of an oxidation process. The H-ion concentration of the bile in the oxygen tube was not materially increased in contrast to the one kept under oil. However, what the actual influence of the ammonia absorption by the bile may be, the loss of CO_2, whether rapid or slow, is in our opinion the main factor in the lowering of the H-ion concentration.

Exposure to air therefore produces an hepatic duct bile and, as already stated, also a gallbladder bile which differs materially from the one present in the rabbit body. We are dealing here with a distinct test-tube artefact. Two points deserve, however, a more detailed inquiry in the future, namely: 1. Is the decrease of the H-ion concentration always of the same rapidity in the hepatic duct and the gallbladder bile of individually different rabbits kept under identical conditions? 2. What is the final P_{H^+} of such biles when exposed to air for 24 hours? Thus far we possess only a few suggestive observations, namely, experimental alkalosis may produce hepatic duct biles in which the H-ion concentration decreases in a somewhat shorter time than in the secretion obtained from animals kept on acid forming or mixed diets. Aside

from the point already mentioned, we call attention to the slight fluctua tions in the P_H^+ of fistular bile in the course of a single experiment. In some instances the gradual increase in the H-ion concentration was mainly the result of frequent collections, which were made necessary by the administration of a cholagogue. On the other hand, a slight increase would be readily explained by the gradual CO_2 depletion of the blood and the disturbance of the alkaline reserve balance, which follows the operative procedures and the restriction of bodily move-ments unavoidable in these experiments. Most of our P_H^+ determina-tions were made by means of the colorimetric method and represent therefore only relative values. For practical purposes it has been suffi-ciently proved that the bile of rabbits, irrespective of its origin, changes its reaction when standing exposed to air and that a P_H^+ usually above P_H 8.4-9.0 may be reached in from 12 to 24 hours. The importance of the observation will be appreciated fully in the paper on the antiseptic effect of bile.

ALKALINE RESERVE OF HEPATIC DUCT BILE

We were able to determine the alkaline reserve as bicarbonate on the amount of CO_2 present in hepatic duct bile by the method of Van Slyke and Cullen.[12] It was impossible to obtain sufficient secretion from the gallbladder, which would have enabled us to study the alkaline reserve and the changes in the H-ion concentration simultaneously. Table 3 shows our findings.

TABLE 3
ALKALINE RESERVE IN HEPATIC DUCT BILE

	Blood Plasma, Volume %	Hepatic Bile Duct, Volume %
Rabbit 1418 (mixed diet)	42.4	116.9 on standing
Rabbit 1801 (mixed diet)	50.3	115.2 under oil 105.7 exposed to air

These data show that the alkaline reserve of hepatic duct bile is more than twice, nearly three times as great, as the one found in the plasma. The average is about 115 volume % of CO_2 found at 0 degrees temperature and 760 barometric pressure. Most of the CO_2 is appar-ently fixed in form of carbonates, but the additional presence of gaseous CO_2, which escapes on standing, is shown by the lower volume in per-

[12] J. Biol. Chem., 1917, 30, p. 291.

centage finding of the specimen of rabbit 1801 when exposed to air. Our plasma bicarbonate figures are lower than those reported by Asada.[13] The differences are probably explained by the fact that we used the heart blood of rabbits which had been operated on, and which had remained in a fixed position on the operation board for at least 6 hours; factors which are known to reduce the alkaline reserve of the blood.

CHANGES OF THE H-ION CONCENTRATION OF THE BILE IN EXPERIMENTAL CONDITIONS

Guided by Nichols's [1] conception that the germicidal property of rabbits' bile in vitro disappears on neutralization with a strong acid (HCl or H_2SO_4) and that therefore the reaction of this secretion governs its destructive influence on bacteria, we conducted tests to confirm the above claims. We furthermore attempted to support his second suggestion, namely, that rabbits in a state of alkalosis produced by a previous injection of sodium bicarbonate are protected against a gallbladder infection. Before one can accept this contention he must show that an experimental acidosis or alkalosis really influences the reaction of the bile. Okada [14] failed to notice a change in the H-ion concentration of fistular bile of dogs fed 200 c c of N/10 HCl solution. On the other hand, we have already recorded the influence of base-forming diets on the biliary secretions of rabbits, a condition which we also attempted to enhance by the use of sodium bicarbonate. We attempted also to solve the next question, namely, is the reverse true? Does the feeding of HCl or a state of experimental acidosis increase the H-ion concentration *in vivo?* The latter procedure gave some suggestive results, but for practical purposes we can probably accomplish in the rabbit the same effect through fasting or the prolonged exclusive feeding of acid-forming diets. A few tests were also made on rabbits which were in a state of hypercholesterolemia.

EXPERIMENTS IN VITRO

Five to 10 c c samples of hepatic duct bile were carefully neutralized with undiluted lactic acid or N/10 HCl. The chemical was added drop by drop, the precipitate which formed under production of gas was redissolved by shaking the tube. A number of tests were also conducted with hepatic bile partially saturated with CO_2. $P_H{}^+$ determinations were made at varying intervals.

[13] Am. J. Physiol., 1919, 50, p. 1.
[14] J. Physiol., 1915, 49, p. 457; 1915-1916, 50, p. 114.

TABLE 4

THE H-ION REACTION OF "CARRIER" BILES

Rabbit No.	Diet and Treatment	Agglutination Reaction of Bile	Serologic Findings of Blood Serum		P_H+	Appearance of Bile and Bacteriologic Findings
			Agglutination	Complement Fixation		
941	Mixed diet, laparotomy	1:10	1:1000	0.05 ++	7.2 (2′) 8.4 (60′)	Light greenish, limpid, ∞ B. typhosus
942	Mixed diet, laparotomy	.....	>1:20,000	0.0005	9.0 (60′)	Light green, limpid, thick wall, ∞ B. typhosus
943	Mixed diet, killed	.....	.....	.....	8.0	Light green, limpid, thick wall, ∞ B. typhosus
944	Mixed diet, killed	.....	1:6000	.....	8.4	Light green with soft stone. ∞ B. typhosus
946	Mixed diet, killed	1:100	1:1000	0.05	8.2	Clear light green bile, wall thick. ∞ B. typhosus
947	Mixed diet	<1:40	1:6000	0.005	8.0	Brownish slimy bile with brownish green débris. wall thick, ∞ B. typhosus
961	Mixed diet, laparotomy	<1.10	1:1000	.....	>8.4 (30′)	Light green, limpid, B. coli
963	Mixed diet, laparotomy	1:10	1:2000	0.05	8.4 (30′)	Light greenish, yellowish slimy sediment, ∞ B. typhosus
671	Mixed diet, laparotomy	1:20	1:1000	.....	>8.4 (30′)	Light greenish bile, sediment, thick wall, B. coli
672	Mixed diet, laparotomy	.....	1:400	0.005	8.3 (5′)	Light greenish with adherent crusty sediment, ∞ B. typhosus
676	Mixed diet, laparotomy	1:800	1:1000	.....	8.2	Purulent, colorless, viscid, wall thick
	Recovered at necropsy	1:20 +++	1:500	.....	7.4	Clear yellowish green, sterile
997	Mixed diet, killed	.....	1:1000++	.....	7.3	Light green, considerable granular sediment, thick wall, ∞ B. para. B
1035	Mixed diet	.....	.....	.....	8.0 (10′)	Light greenish, limpid, ∞ B. typhosus
1048	Mixed diet, laparotomy	>1:1000	>1:20,000	<0.005	8.4 (30′) (+0.1)	Light greenish, limpid, very little sediment, wall injected, ∞ B. typhosus
1053	Mixed diet, laparotomy	1:60	1:10,000	0.005	8.4 (30′) (—0.1)	Colorless, sand-like yellow débris wall adhesions, ∞ B. typhosus
1054	Mixed diet, laparotomy	1:20	1:4000	0.01	8.4 (30′)	Clear light green bile in large dilated gallbladder, ∞ B. typhosus
1056	Mixed diet, laparotomy	1:200	1:8000	0.005	8.2 (30′)	Dark green viscid bile, sterile
1057	Mixed diet, laparotomy (coccidiosis, few patches)	1:200	>1:20,000	0.005	8.4 (30′)	Purulent, colorless, slimy bile with considerable sediment, ∞ B. typhosus
1059	Mixed diet, laparotomy	1:20	1:6000+++	0.005	8.4	Light greenish bile with greenish sand-like sediment, wall adherent, abscesses, ∞ B. typhosus
1059b	Mixed diet, laparotomy	<1:10	1:1000	0.005	8.4	Light greenish limpid bile with considerable sand-like sediment, ∞ B. typhosus
1124	Oats, killed	.....	1:20,000	.....	7.5	Purulent, slimy with considerable sediment, wall thick, ∞ B. typhosus
1129	Oats, killed	.....	.....	.....	7.0 (2′)	Purulent, slimy with considerable sediment, wall thick, ∞ B. typhosus
1131	Oats, killed	.....	1:10,000	.....	7.5	Greenish, very slight sediment, ∞ B. typhosus
1135	Oats, killed	1:600	.....	.....	7.7 (5′)	Purulent slimy, slightly colored, ∞ B. typhosus
1157	Oats and starvation	.....	.....	.....	7.6	Purulent, colorless, sand-like, greenish sediment, ∞ B. typhosus
1160	Oats, severe infection	1:10-20	1:2000+++	.....	7.5 (5′)	Slimy, colorless, sand-like sediment, 11,800,000 B. typhosus per 1 c c
1163	Oats, Na_2CO_3	<1:10	1:1000	.....	7.8	Light green, limpid, sterile, staphylococcus 100 per c c
27 Rabbits	Average....	1:200 (1:10-1000)	1:400-1:20,000	.....	8.0	

* If not specifically stated the reactions were determined immediately on collection.

From the data thus collected it became apparent, that neutralization changes only temporarily the H-ion concentration of hepatic duct bile when exposed to the air. In some biles the H-ion concentration decreases progressively in 24 hours until a reaction identical with the original specimen is reached. Preservation of bile under oil delayed this decrease and in most instances the original P_H^+ was not attained. These observations, therefore, indicate that neutralization does not prevent a bile specimen from changing its H-ion concentration. Invariably such fluids exposed to the air reached a reaction which must be considered unfavorable for the growth of B. typhosus and other organisms.

In a few experiments hepatic duct bile of a P_H^+ of >8.8 was partially saturated with CO_2, which increased the H-ion concentration to P_H^+ 6.8-7.0. Exposure to the air in a water bath caused a rapid decrease, and in 24 hours the original P_H^+ was reached. Stratification with oil naturally retarded the return to a low H-ion concentration.

EXPERIMENTS IN VIVO

A small series of rabbits was kept on different diets. Common duct fistulas were made on these animals. The H-ion concentration of the blood was determined by the dialysis method of Levy, Rowntree and Marriot;[15] the the alkaline reserve, R_{PH}, according to the procedure of Marriot; and the reaction of the urine by the colorimetric method. Collections of hepatic duct bile before and after the feeding of varying amounts of HCl or intravenous injection of $NaHCO_3$ were made, and the reactions determined.

In the course of these tests it became evident that final conclusions cannot be drawn from the limited number of experiments. In most instances striking changes in the H-ion concentration of the hepatic bile were not demonstrable. In only two rabbits (692 and 1118), which had been nourished on a mixed oat and hay or cabbage diet respectively for one month, there was recorded a slight decrease in the P_H^+ of the hepatic duct bile during the 2 hour period following the administration of HCl by the stomach tube. In one animal (1118) the R_{PH} of the blood had fallen from 8.6 to 7.8 and the urine also turned acid. In another rabbit (1031 b) the H-ion concentration of the blood decreased following the use of HCl. The R_{PH} remained unaltered, and consequently the influence of the P_H^+ of the hepatic bile was not felt in the two periods following the feeding of HCl, or was so slight that our crude methods failed to record it. We desire also to call attention to the fact that the literature records cases of experimental acidosis in rabbits, in

[15] Arch. Int. Med., 1915, 16, p. 389.

TABLE 5

ACCORDING TO THE FOLLOWING AUTHORS ONE KILOGRAM OF ANIMAL WEIGHT SECRETES IN 24 HOURS

	Hepatic Duct Bile		Remarks
	Rabbit	Guinea-Pig	
Heidenhain (1883)	136.84 c c	175.84 c c	Gallbladder fistulae (?)
Mann (1918) (4 animals each)	56.84 c c	130.29 c c	Hepatic duct fistulae
Neilson and Meyer (1920)	90.34 c c (17 animals)	154.27 c c (9 animals)	Hepatic duct fistulae
Average	71.34 gm.	153.46 gm.	

TABLE 6

THE RELATION OF BILE SECRETION TO THE BODY AND LIVER WEIGHT OF RABBIT AND GUINEA-PIG

	Rabbit	Guinea-Pig
1. Average weight: Heidenhain	1525.8	518
Mann	2158.8	561.8
Neilson-Meyer	2506.6	899.2
2. Fresh bile per kg. body weight in one hour:		
Heidenhain	5.070	7.32
Mann	2.36	5.42
Neilson-Meyer	3.76	6.42
3. Proportion of liver weight to body weight:		
Heidenhain	1:33.5	1:27.3
Mann	1:30.3	1:18.1
Neilson-Meyer	1:31.1	1:22.09
4. Fresh bile per kg. of liver per 1 hour:		
Heidenhain	169.3	185.5
Mann	84.9	130.6
Neilson-Meyer	122.1	134.5

TABLE 7

THE INFLUENCE OF DIET ON THE RATE OF BILE FLOW PER KILOGRAM WEIGHT FOR RABBITS, GUINEA-PIGS AND RATS

Number of Animals Tested	Diet and Treatment	Average Weight, Gm.	Average Weight of Liver, Gm.	Average Amount of Bile Collected in the First Hour, C c	Average Amount of Bile Collected During 6 Hours, C c	Average Estimated Total of Bile for 24 Hours, C c	Average Capacity of Gallbladder C c	Average Percentage of Bile Secreted in 24 Hours, Which the Gallbladder Will Hold
6 rabbits	Mixed	2,725	90.5	10.09	56.1	218.4 (80.1 per kg.)	3.1	1.4
5 rabbits	Mixed and cholagogue	2,385	73.1	11.0	42.5	156.1	2.0	1.5 (?)
2 rabbits	Fasting	1,990	52.0	5.65	33.7	.....	...	...
4 rabbits	Oats	2,650	76.0	9.75	67.03	279.9 (105.62 per kg.)	...	...
7 rabbits	Cabbage	2,145	75.25	9.69	45.7	182.9 (85.26 per kg.)	1.8	0.96
2 rabbits	Carrots	1,982.5	62.0	10.4	51.75	223.5	2.0	0.7
2 rabbits	Oats and Na_2HCO_3	2,925	112.0	12.0	64.2	256.8	2.8	1.2
9 guinea-pigs	Mixed	899.2	40.7	10.95	.....	138.82	1.5	0.96
2 rats	Mixed	298.5	.....	0.97	1.3-5.1	23.28	...	...

which the changes of the buffer values of the blood are inconstant, and it is reasonable to suspect that unless the blood alkalies are seriously depleted no appreciable change will be noted in the reaction of the biliary secretions.

It is even more difficult to provoke changes by means of sodium bicarbonate in the H-ion concentration of the hepatic duct bile of rabbits fed with acid-forming diets. The alkali reserve can be readily increased, but in our few experiments this was not followed by any noticeable decrease in the H-ion concentration of the bile. Kurijama and also McClendon, von Meysenberg and Engstrand [17] noted the effect of diet on the alkaline reserve of the blood of rabbits, which is also indicated in our observation by the rather low R_{PH} of several rabbits. The injection of 1 to 2 gm. of $NaHCO_3$ increased the R_{PH} slightly, but the alkali depletion (acid diet, narcosis, etc.) was apparently so great that P_{H^+} of the urine was only slightly decreased. In one instance it was even increased. And again, in the oat fed rabbits, a marked holding back of alkalies took place. It may therefore be necessary to give more than 2 gm. of $NaHCO_3$, just as is the case in pathologic conditions in order to produce their elimination with the urine or with the bile. The titrable alkalinity also showed no noteworthy increase. It should be recalled that only immediate determinations of the H-ion concentration will give P_{H^+} readings which are closely analogous to those of the blood. In a thoroughly alkalinized rabbit (1200) the rapid decrease in the H-ion concentration on standing, and the low final reaction has already been commented on and is undoubtedly the best proof that feeding or injection of alkalies may influence the reaction of bile. How far a moderate alkaline therapy may change the reaction of gallbladder bile cannot be determined satisfactorily on rabbits with common duct fistulas. But we have already recorded in table 2 that 3 rabbits in the state of experimental alkalosis had gallbladder biles with an average P_{H^+} reading of 7.6. A similar H-ion concentration can possibly be obtained more readily and with less danger in feeding a base-forming diet, like cabbage and carrots. We will have occasion to consider this point repeatedly in other papers of this series. Judging from a few controlled experiments, a state of acidosis develops during starvation which influences very little the reaction of the hepatic duct bile. This corresponds with the observations of

[16] J. Biol. Chem., 1918, 33, p. 215.
[17] Ibid., 1919, 38, p. 539.

Asada.[13] The cystic bile is, however, always distinctly acid. A reduction of the plasma bicarbonate concentration is in our experience, so far as the rabbit is concerned, followed by a high H-ion concentration of the cystic bile.[18] The hepatic duct bile remains unchanged. The factors which are responsible for the difference can be determined only by further extensive experimentation.

Experimental cholesterolemia has no influence on the reaction of the hepatic duct bile. The low P_H^+ of the cystic bile of several rabbits is probably the result of fasting or slight under-nutrition. The majority of the animals took the lanolin or brain-carrot mush rather reluctantly, refusing it frequently for several days. The blood of the successfully cholesterolized rabbits contained over 10 times the amount of cholesterol ordinarily found (Bloor[19] — 42 mg. per 100 c c). Hepatic duct bile never contained cholesterol in amounts exceeding 100 mg. per 100 c c of bile.

THE RESULTS WITH GUINEA-PIG BILE

In the course of our experiments we tested 47 gallbladder and 10 hepatic duct bile samples of healthy guinea-pigs kept on a liberal diet of hay, oat and greens. In accordance with the findings of Nichols, we found this secretion to be alkaline to phenolphthalein; the titrable alkalinity varied from 0.43 to 1.8 or 0.154 to 0.720 mg. of HCl per 1 gm. of bile. The P_H^+ was always above 7.0 and H-ion concentration decreased rapidly in a similar manner, as determined for rabbit bile on standing exposed to air. It was practically impossible to collect the hepatic duct bile without exposure to the air. The 2 successful collections gave readings of P_H^+ 7.7 and 7.8. When compared with the cystic bile of the same animals, we found that the P_H^+ of the latter was only 7.2 (normal—P_H^+ 7.55). These figures suggest, but do not prove, that hepatic duct bile is slightly more alkaline than cystic bile. In the majority of instances, however, the cystic bile differed from the hepatic duct bile neither in its reaction, color nor physical consistency. In the animal body the H-ion concentration is probably identical with that of the blood. Guinea-pigs suffocated or moribund with a blood P_H^+ of 7.0-7.2 due to an accumulation of carbon dioxide gave gallbladder specimens which were neutral or slightly alkaline (P_H^+ 7.0-7.1) when tested

[18] Hirsch: Jour. Am. Med. Assn., 1920, 75, p. 1204.
[19] J. Biol. Chem., 1916, 24, p. 227.

immediately. The data available illustrate, however, the progressive decrease on standing.

THE RESULTS WITH DOG, CAT, GOAT, RAT, MONKEY, OX, SHEEP AND PIG BILE

The measurements of the H-ion concentration of the hepatic duct and cystic bile of a series of different animals gave varying results. Two facts were demonstrated: 1. The reaction of the gallbladder bile is always more on the acid side and varies more than the hepatic duct bile. 2. Both secretions change their reaction on standing. The decrease in the concentration is apparently more rapid, and a lower $P_H{}^+$ value, namely 8.2-8.4, is reached in dogs (five) fed and injected intravenously with sodium bicarbonate. Our findings corroborate fully the observations of Okada,[14] who states that it was necessary to keep dog bile free from air, otherwise the value of the H-ion concentration is found to be subject to change.

In connection with our problem on the antiseptic effect of bile, it was of particular interest to record the progressive decrease in the H-ion concentration of gallbladder bile of oxen. Some samples of bile collected aseptically from the gallbladder in from 1 to 2 hours after death and stored in cotton stoppered test tubes, reached a H-ion concentration of $P_H{}^+$ 8.4-9.0. The entire bacteriologic literature dealing with the antiseptic and the inhibitive effect of ox bile has apparently overlooked this phenomenon. Invariably heated sterilized ox bile collected in San Francisco abattoirs has a reaction which certainly cannot be considered ideal for the growth of organisms of the typhoid-paratyphoid group, and yet no attempts have been made to adjust this important factor. Is it not possible that some of the reports and the various contradictory statements relative to the germicidal effect of ox bile can be ascribed to this unrecognized factor? Is perhaps the reduction of the inhibitory effect of ox bile on B. typhosus by the addition of glycerol (E. E. Ecker[21]) explained by the fact that impure glycerol is slightly acid and could therefore improve an alkaline bile specimen as a culture medium. All of these questions will be answered in the next paper, but it is sufficiently manifest that attention should be called to the alkaline reaction of old ox bile and to the possibility that chemical transformation takes place more readily in such an environment than when the medium is neutral.

[21] J. Infect. Dis., 1918, 22, p. 95.

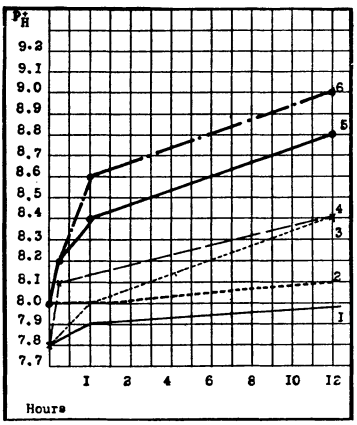

Chart 1.—Change in H-ion concentration of hepatic duct bile on standing at room temperature. 1, rabbit 1481—collected under paraffin oil sealed with rubber stopper; 2, rabbit 1481—corked with rubber stopper, a few bubbles at the top; 3, rabbit 1182—partially filled tube, sealed with rubber stopper; 4 rabbit 1182—partially filled tube corked with cork; 5, rabbit 1200—exposed to air, plugged with cotton; 6, rabbit 1200—exposed to CO_2 free air in desiccator.

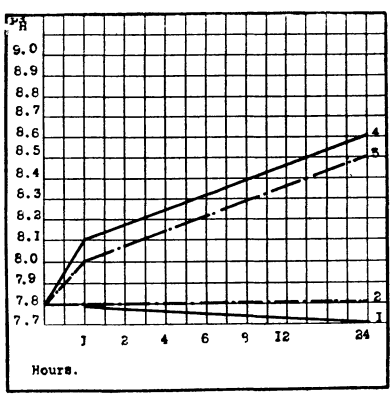

Chart 2.—Change in H-ion concentration of gallbladder "carrier" bile on standing at room temperature. 1, rabbit 1625—bile containing 22,500,000 B. typhosus per c c kept under sterile condition in the removed gallbladder; 2, rabbit 1630—bile containing 80,000,000 B. typhosus per c c kept under sterile condition in the removed gallbladder; 3, rabbit 1630—bile in small tube closed with cotton stopper then incubated and found sterile in 4 days; 4, rabbit 1625—bile in small tube closed with cotton stopper, then incubated and found sterile in 15 days after removal.

The only gallbladder bile specimens which remained unchanged on standing or showed after 24 hours an increased H-ion concentration were derived from sheep. In tightly corked tubes the P_{H^+} decreased from 7.2-6.9. This indicates that there is not only no loss of CO_2, but there also is probably a formation of lactic acid on standing. Further tests will tell whether this increase in the H-ion concentration is characteristic for gallbladder bile of sheep.

Bile samples obtained by fistula from rats behaved like liver bile and were decidedly alkaline. The gallbladder bile of monkeys was acid or alkaline; in one instance the hepatic duct bile was found alkaline.

In connection with our colorimetric measurements of the H-ion concentration of these bile specimens we conducted a series of titration tests. We can in general confirm the observations of Jolles [22] Chittenden and Albro [23] and others. The cystic bile of oxen, sheep, goats, pigs, cats, dogs and monkeys is acid to phenolphthalein. From 0.22 to 0.76 mg. of KOH or NaOH were required to neutralize the acid salts of 1 gm. of the different specimens. This acidity varied considerably for the individual animal of the species, and it is apparent that the same factors cited for the differences in the H-ion concentration, namely, the general health of the animal and its diet, influence the titrable acidity or alkalinity. To these two factors we desire to add a third, namely, the time period after death elapsing between the collection of the biliary secretion from the gallbladder gradually becomes more acid. Those exposed to the air in test tubes become more alkaline. The data on which these statements are based was obtained in titrating the specimens as fresh as possible, at least within two hours after death, and immediately after their collection from the gallbladder. Adherence to this principle may in part explain the lower acidity noted in our series in comparison with findings reported by Chittenden and Albro [23] and those of Jolles.[22]

The titration method also shows that the hepatic duct bile is different from the gallbladder bile. Invariably the former is very slightly acid to phenolphthalein; the majority of the 1 gm. bile samples required less than 0.1 mg. of NaOH for neutralization. These specimens were uniformly alkaline to litmus.

RESULTS WITH HUMAN BILE

As we found that the reaction of gallbladder bile retained in the rabbit body for some time was subject to change, we considered it

[22] Arch. f. d. ges. Physiol., 1894, 67, p. 1.
[23] Am. J. Physiol., 1898, 1, p. 307.

unnecessary to study specimens collected from necropsy examinations. Aside from a few samples obtained from gallbladders after cholecystectomy, we were fortunate in having a case of biliary fistula in the wards. The biliary secretion was collected for a number of days, and the reaction of the same was determined by the titration and by the colorimetric method. The patient felt more comfortable, and the liver bile was less mucoid, when sodium bicarbonate (6 gm. daily), Carlsbad salts and magnesium sulfate were freely administered. This alkaline therapy has to be taken into consideration in judging the reaction of the bile, which was always alkaline to litmus; 1 gm. of bile required on the average 0.72 mg. of HCl to neutralize the mono- or di-basic phosphate salts, using lacmoid as an indicator. As a rule, the clear, light greenish fluid was either neutral to phenolphthalein, or 0.04 to 0.08 mg. of NaOH were necessary to neutralize the specimen using the same indicator. The P_{H^+} was usually 8.0 and increased on standing to 8.6. Heated specimens always had a P_{H^+} above 8.4. The latter procedure was necessary to insure sterility of the biliary fluid, which contained a few paracolon bacilli and B. coli aerogenes. The few gallbladder specimens obtained from cholecystectomy cases were alkaline to litmus. The H-ion concentration was low; the range extending from P_{H^+} 7.7-8.6. We have the impression that the specimens were actually liver bile recently discharged into the gallbladder. The bile was light colored, limpid and of low concentration.

In general, our findings agree with those already published. Pisenti,[24] Brand,[25] Copeman and Winston[26] found human fistula bile to be neutral or faintly alkaline; Fränkel and Krause,[27] Toida[28] and others state that the majority of human gallbladder biles were neutral, a few alkaline, but never acid to litmus.

THE RESULTS WITH GALLBLADDER BILE OF RABBIT TYPHOID OR PARATYPHOID CARRIERS

In examining the gallbladder bile of "carrier" rabbits, we employed the methods outlined in the previous paragraphs. Since 1917 more than 50 specimens have been carefully tested, but only an unselected number of 27 are tabulated in table 4. We found that "earrier biles" differed in no respect from those obtained by fistulas, the P_{H^+} being on the aver-

[24] Arch. Med. Ital., 1890, 14, p. 13.
[25] Arch. f. Physiol., 1902, 90, p. 491.
[26] J. Physiol., 1889, 10, p. 213.
[27] Ztschr. f. Hyg. u. Infectionskrankh., 1889, 32, p. 97.
[28] Arch. f. klin. Chir., 1913-1914, 103, p. 407.

age 7.5 immediately on withdrawal from the laparotomized chloroformed animals. In not one instance did we find a bile specimen in which the P_{H^+} was below 7.0; in most instances the P_{H^+} was 7.3-7.6 (normal P_{H^+} 7.22). On standing these samples usually decreased in a shorter time than normal hepatic duct bile and reached P_{H^+} 8.0 within 1 to 2 minutes and above 8.6 in less than one hour. Beckwith [29] made the same observation. Immediately tested all specimens were alkaline to litmus and were either alkaline (0.1) or neutral or very slightly acid to phenolphthalein. In consistency the biliary secretions were limpid, clear and light greenish. These were at times free from pigment, with a sand-like, yellowish-green granular carbonate sediment (up to ⅓ of the total fluid bulk) and microscopically had a dĕbris which consisted of leukocytes, epithelial cell and masses of bacteria. The samples rich in carbonate débris, as is to be expected, changed their reaction rapidly to a low H-ion concentration. Others were slimy and pus-like in character and retained their acidity for a longer period than the clear limpid fluids; the H-ion concentration either remained stationary or increased slightly. This phenomenon is shown in chart 2, curve 2. A carrier bile rich in exudate behaves, therefore, in a somewhat similar manner to spinal fluids derived from meningococcic meningitis (Levinson [30]). It is not unlikely that the mechanism underlying the slow decrease in the H-ion concentration is the same, namely, lactic acid formation due to a destruction of cells on standing. However, owing to the rather small number of purulent carrier biles studied, we are not prepared to offer this suggestion as a final, conclusive statement.

The H-ion concentration of carrier bile stands apparently in close correlation with the physiologic activities of the mucous membrane of the gallbladder. Thickening of the wall with signs of an inflammatory reaction always revealed a limpid bile which behaved similarly to hepatic duct bile. The gallbladder of a "carrier" animal has apparently lost its concentrating function and therefore fails to change the reaction of its content. On recovery from the carrier state, which leads to sterile bile samples and which is accompanied by an increased viscosity and pigmentation, the H-ion exponent returns to the normal variant P_{H^+} range from 6.8 to 7.6. As determined repeatedly, the mere presence of bacteria in the gallbladder bile does not influence the H-ion concentration. A rabbit, for example, in a fasting condition with a typhoid focus in the

[29] Thesis, 1920.
[30] Cerebrospinal Fluid, 1919, p. 142.

liver, which constantly discharges B. typhosus into the bile, but without involvement of the gallbladder wall, may give a P_H^+ reading of 6.4-6.8 plating of the biliary fluid, may demonstrate millions of viable bacteria. It will be our object in the next paper to consider the relationship of the reaction of the standing carrier biles to the progressive sterilization from B. typhosus. Early in our studies we noted that irrespective of the low H-ion concentration, the bacteria present in carrier bile may remain viable for more than 20 days. These observations cast considerable doubt on the conception that the reaction of the bile is the most important factor in the development of a carrier state in rabbits. This drift of thought prompted the diversified studies given above, which in our opinion have shown that in all probability the H-ion concentration of the gallbladder is most suitable for the development of bacteria of the typhoid-paratyphoid group. With the progress of the infection, when changes in the gallbladder wall ensue, the H-ion concentration becomes more or less identical with the one of the blood.

We were unable to correlate the presence of agglutinins in carrier biles with the reaction of the same specimen. Most of our samples were kept in the ice-chest or at room temperature for at least 24 hours before they were used for serologic tests. Their reaction was constantly above P_H^+ 8.0; low or high agglutination tests were encountered. The H-ion concentration, therefore, cannot be considered the factor which governs the agglutination reactions of carrier biles.

DISCUSSION

The reaction of the bile of laboratory animals is treated only in a few scattered statements of the literature. Unfortunately, these notes are not strictly comparable on account of the variety of methods, which are used for the determination of the reaction. In most instances no distinction is made between the hepatic duct and the cystic bile.

Chittenden and Albro [23] measured the alkalinity of rabbit bile flowing directly from the liver through a fistula and not coming in contact with the gallbladder. They found that it had an alkalinity of 2.5 mg. HCl per gm., using phenolphthalein as an indicator. There was no measurable acidity, and these workers therefore concluded that this bile may contain an alkali as strong as sodium carbonate. Nichols [1] titrated his bile samples using phenolphthalein and lacmoid as indicators and noted a neutral or 3 to 6.0 alkaline reaction in rabbit bile. Aside from Quagliariello,[31] who reported the H-ion concentration of gall-

[31] Rendiconti Acc. dei Lincei, 1911, 22, p. 302.

bladder bile of the rabbit to vary from P_{H^+} 6.4 to 7.9, no records of similar determinations by modern methods are available. Apparently no attention has been paid to the changes occurring on standing, and we are therefore unable to establish definitely from the data collected the normal reaction of the liver and of the gallbladder bile of rabbits. Neither could we find statements made with regard to the diet and the general conditions of the animals, which were used for the determinations of the bile reactions.

Our findings as a whole support the contention of Chittenden and Albro.[23] The fistula bile of rabbits is alkaline to phenolphthalein and this alkalinity is probably due to carbonates, as base-forming diets increase noticeably the average titrable alkalinity. The H-ion concentration of the hepatic duct bile varied between P_{H^+} 7.6-7.9; it was never below P_{H^+} 7.5. We are fully convinced that if technical difficulties could be overcome, the reaction would be found to correspond with the one determined for the blood, which according to Hasselbalch [32] is P_{H^+} 7.33, (at 38 degrees) and for the rabbit P_{H^+} 7.65 (Dragstedt [33]). A well balanced equilibrium apparently exists between the CO_2 tension of the blood and the biliary secretions. This explains our difficulties in changing the H-ion concentration of hepatic duct bile by various diets, HCl feeding or intravenous injection of $NaHCO_3$, even when the titrable acidity or alkalinity differed from the normal average. Misleading values of the H-ion concentration can, however, be obtained when the bile samples are exposed to the air for some time; low concentrations are regularly recorded. We found that the P_{H^+} ranged from 8.2 to >8.6; readings of over 8.2 being sometimes obtained 10 minutes after removal from the animal body. Our tests have shown that in the first place these changes follow the escape of CO_2; then possibly the absorption of ammonia.

Gallbladder bile of rabbits was found in a large series to be acid to phenolphthalein. Colorimetric determinations, on the other hand, have shown that these biles can be acid, neutral or alkaline. The P_{H^+} range extends from 5.7-7.7. The variability of the reaction exists in individual rabbits and seems to be connected with the general health and the diet of the animals. Fasting, disease and acid-forming diets and HCl feeding produce an acid reaction in contrast to the alkali increasing effect of base-forming diets. Also the cystic bile changes on standing,

[32] Biochem. Ztschr., 1911, 30, p. 317.
[33] Jour. Infect. Dis., 1920, 27, p. 459.

perhaps less rapidly and inconstantly than the hepatic duct bile. The final H-ion concentration of such samples does not decrease below P_{H^+} 8.0-8.2. This is particularly true when the specimens have been retained in the gallbladder for at least 2 to 6 hours. Concentrated, viscid biles are also less subject to changes than the more dilute limpid specimens. It will be clear from this statement that the cystic bile of rabbits never reaches even in vitro a reaction which is destructive to bacteria, for example, B. typhosus. In most instances in vivo the H-ion concentration of the gallbladder bile will be P_{H^+} 6.8-7.0. Schoenholz and Meyer have shown that this reaction in salt-free buffered broth favors the optimum growth of B. typhosus. In the light of all these findings it is not logical to accept Nichols's conclusions based on in vitro experiments that rabbit bile is antiseptic on account of its alkalinity.

As far as we have been able to survey the literature, no one has as yet determined the H-ion concentration of rabbit carrier bile. We found that such biles behave like the hepatic duct specimens or biologic fluid rich in leukocytes. On standing they either show decreasing, stationary or increasing H-ion concentrations. In vivo the reaction is probably identical with the one characteristic for the blood of the individual rabbit and most suitable for bacterial growth. In vitro a reaction is sometimes reached which must be considered unfavorable for the bacteria of the typhoid-paratyphoid group. Germicidal properties develop only when other factors have made their occurrence, as will be discussed in detail in the next paper.

Nichols first called attention to the strongly alkaline reaction of the guinea-pig bile. As far as titrable alkalinity is concerned, we can fully support his findings; however, the H-ion concentration is high for freshly measured biles and decreases rapidly on standing. In the guinea-pig body the reaction is identical with the one of the blood.

Our H-ion concentration measurements on dogs, cats, goats, oxen, sheep, pigs, rats, monkeys and human bile correspond with those reported by Okada [14] and Quagliariello.[31] The reaction of the bile from the gallbladder and from the hepatic duct is different. The reaction of the former is more variable and inclines toward the acid side. The general condition and diet of the individual of the same species are in part responsible for this variability. And again, the average titrable acidity or alkalinity varies for different species. With the exception of sheep bile, most of the gallbladder biles exposed to the air show a decrease in the H-ion concentration on standing. The escape of CO_2

is responsible for this change and is particularly striking in ox bile. This fact should be carefully considered in future studies on the inhibitive and antiseptic affect of this secretion.

The hepatic duct biles of dogs, cats and one human case of biliary fistula were slightly alkaline when tested immediately on withdrawal from the body; they also changed on standing. A carefully adjusted equilibrium between the H-ion concentration of the blood and the liver existed apparently also in these species, just as we discussed it for the rabbit and guinea-pig. The liver bile of dogs which are in a state of experimental alkalosis shows a more rapid fall in the H-ion concentration than the hepatic duct bile of the normal.

The numerous experimental bile collections made in the course of our study, supply a number of physiologic data, which have not only a bearing on certain phases of our problem but contribute to the knowledge of the function of the gallbladder and the importance of the biliary secretions in general. These facts are briefly enumerated in the following paragraphs.

RATE OF BILE-FLOW AND THE INFLUENCE OF CHOLAGOGUES

Any one working with animals possessing a biliary fistula is at once struck by the fact that the small herbivorous animals, rabbits and guinea-pigs, secrete considerably more bile in a given time interval than the omnivorous and carnivorous species. When comparing our quantitative findings, recorded in tables 5 and 6, it will be seen that the guinea-pig secretes usually more than twice the amount of bile in 24 hours that the rabbit does. Our figures are based on a considerably larger series of animals than those published by Heidenhain [34] in 1883 and by Mann [35] in 1918. Their estimates are not strictly comparable with our own for these reasons: Mann made the collections from animals while under an anesthetic, which in his opinion probably decreased the secretion. The data presented by Heidenhain were presumably collected from the work of Bidder and Schmidt [36] and were made in a period (report published 1852) when anesthetics were not regularly used in animal experiments. It is our custom to let the animal completely recover from the ether before the rate of bile flow is estimated. We possess, however, sufficient data which show that this operative procedure had but a slight influence on the output of bile per hour. In contrast to Mann, we

[34] In Hermann's Handb. d. Physiol., 1883, 5, Pt. 1, pp. 249 and 412.
[35] New Orleans Med. & Surg. Jour., 1918, 71, p. 80.
[36] 1852, p. 191.

determined the bile secretion for at least 6 hours and estimated our total daily output from this figure. We have gradually become accustomed to consider the average hourly output as 10 c c for rabbits and guinea-pigs. There are individual differences, and temporary reflex inhibition or dislocation of the cannula may influence the total collection. According to our figures, the daily output of bile represents $\frac{1}{8}$ to $\frac{1}{10}$ of the total body weight of the animal (table 7).

The rate of flow is not perfectly uniform. Some animals show a decided decrease in secretion toward the 5th and 6th hour of the experimental period, others a marked increase. These differences are more or less individual, and their cause cannot be easily explained. Improperly secured and restless animals increase by their bodily movements the rate of the bile flow. The diet changes the total 24-hour output. A strict oat diet with a liberal amount of water apparently stimulates bile secretion. The base-forming diet, like hay, cabbage and carrots, produces on the average 80 to 90 c c of bile per 1 kg. weight of rabbit in 24 hours. It is not unlikely that the findings of Bidder and Schmidt [36] are partially explained by the fact that their experimental rabbits were kept on a liberal hay and oat diet. Our observation corroborates the statement made by Abderhalden [37] and suggests that in future determinations of the rate of bile flow proper attention should be paid to the diet of the animals. Unfortunately only two experiments on fasting rabbits are available for comparison. In both rabbits the rate of bile flow was so markedly decreased that even the use of cholagogues failed to increase the hourly output to a noteworthy degree.

As already stated, the reflex inhibition influenced the rate of bile flow in the dog, cat, goat and monkey. Accurate determinations of the amount of bile secreted are therefore impossible.

In the 2 rats successfully operated on and kept under observation for from 12 to 24 hours, the flow of bile was irregular; in one animal the hourly output at the beginning of the experiment was more than 1 c c, in the second it was less than 0.5 c c. The total amount of bile collected in 24 hours represents about one-ninth to one-tenth of the body weight.

In a case of human biliary fistula in which the patient received sodium carbonate and Carlsbad salt, the hourly rate of bile flow fluctuated between 13.2 to 67.4 c c. The total secretion estimated on collections made for 6 hours on 5 successive days averaged 885 c c for 24

[37] In Ellenberger and Scheunert: Lehrbuch d. vergleich. Physiol. d Haussäugetiere, 1910, p. 267.

hours. The secretion was never continuous and was clearly correlated with the bodily movements. According to figures published by Copeman and Winston [38] (779.6 c c for 24 hours), Pfaff and Balch (88) (525 c c) and others our averages appear rather high. It is not unlikely that the alkaline therapy may in part be responsible for this increased rate of bile flow.

The effect on the bile flow of ox bile feeding and the intravenous injection of sodium taurocholate in rabbits was observed in the course of several experiments. The feeding of from ½ to 1 ounce of ox bile in two experiments failed to increase the rate in the 3 to 4 hours subsequent to its administration. Practically the total amount of bile introduced was found in the stomach at necropsy. Intravenous injection of from 200 to 500 mg. of sodium taurocholate produced, as is indicated in table 3, an immediate but temporary stimulus in the rate of bile flow. The hourly average of 5.6 c c increased in one instance to 13.5 c c and in another from 2.8 c c to 9.3 c c. As a rule, the familiar cholagogue action of the taurocholates was spent within the next 2 to 4 hours, depending on the amount injected.

COLOR OF THE BILE

In the course of our experiments we found that the changes in the color of the bile of different species of animals may serve as a valuable guide in judging the age of a specimen. It appears therefore necessary to give a brief description of the various color changes developing a result of oxidative or reductive processes. Rabbit bile secreted from the common duct is clear and has a light green color, which usually deepens to a smaragadine green color on standing. Exposed to the air the sterile fluid acquires in the course of a week a slightly brownish shade. The green gallbladder bile changes more rapidly to a deep brown color. After 2 days usually the bilirubin is transformed to hydrobilirubin. When protected from oxidizing processes by stratification of the sample with paraffin oil, the color remains unaltered for at least 2 weeks.

The hepatic duct bile of the guinea-pig is a light golden yellow color which on exposure to the air becomes a dull green or even a light brown. Cystic bile usually is of the same color; in some animals the secretion may be entirely colorless. A similar color scale can be noted in rat bile.

[38] J. Physiol., 1889, 10, p. 213.

The bile of dogs, cats, monkeys and man secreted from the liver is a light brownish yellow, sometimes with a greenish tinge which changes on standing to a dull brown or golden brown. The gallbladder bile of these species, including pigs, may vary from an olive green to a dull reddish brown color, depending entirely on the age of the specimen.

Ox, goat and sheep bile is olive green when fresh, but changes to a dull brown when exposed to the air. Retained in the gallbladder for more than six hours, a similar change is noticeable. The alterations in the color are usually accompanied by definite changes in the reaction of the fluid. This important fact has already been discussed in detail.

THE FUNCTION OF THE GALLBLADDER AND THE DIFFERENCES BETWEEN HEPATIC AND CYSTIC BILE

In the course of a study of the biliary secretions as a medium for the development of bacteria it becomes apparent that certain differences exist between the bile collected from the hepatic duct and the one procured from the gallbladder. Irrespective of the fact that our knowledge concerning the function of the various biliary secretions and particularly of the gallbladder is meager, a brief review of the essential positive findings, as far as they concern our problems, is herewith attempted.

In the light of the work of Mann,[35] Rost [39] Rous and McMaster [40] and others it must be recognized that the gallbladder has a mechanical and probably a chemical function. The gallbladder influences the flow of bile and acts as a current regulator of the hepatic duct bile. Observations made by Rost,[39] Eisendrath and Dunalvy [41] show that usually all the ducts outside the liver dilate after the removal of the gallbladder. The work of these investigators indicates, that at least in certain species of animals, the gallbladder has a definite function. Okada [14] has demonstrated rhythmic contractions in the gallbladder which increase during the height of digestion and after the administration of acids. Mann [35] noted inactivity of the sphincter of Oddi in animals without a gallbladder. The secretions of the bile in such species is continuous. In animals with active sphincters, a gallbladder is necessary to regulate the bile secretion and discharge and to prevent the fluctuations in the intraduct pressure.

[39] Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1913, 26, p. 710.
[40] Jour. Exper. Med., 1920, 32, p. 249; Proc. Soc. Exper. Biol. & Med., 1920, 17, p. 159.
[41] Surg., Gynec. & Obst., 1918, 26, p. 110.

The mechanism controlling the action of this viscus is, according to Meltzer,[42] under nervous control. Disturbances of the law of contrary innervation, in his opinion lead to pathologic stasis. Fasting, starvation or irregular partaking of food does not supply the necessary peptones, which cause the bladder reflex to discharge the bile. In rabbits we have noted the influence of starvation in producing stasis of bile in the gallbladder.

Our observations also confirmed the recent studies of Mann, which showed that the gallbladder of no species of animal is capable of holding more than from 2 to 5% of the total amount of bile secreted in 24 hours. The function of the gallbladder as a reservoir in the same sense as the urinary bladder is not entirely justified, even if we admit that periodical storage in the gallbladder has probably a certain protective advantage to the intestines or some of its functions.

The bile collected from the gallbladder is more concentrated than that withdrawn from the hepatic duct. Hammersten [43] found from 1.11 to 1.19% solids in the hepatic and from 8 to 10% in the cystic bile. This difference is the result of the concentrating function and said to be due to an absorption of water, but it is now considered to be caused by the addition of material produced by the cells lining the outer biliary passages. In this way mucous material (mucin, phosphoprotein) and some cholesterol are added to the hepatic bile. Their functional significance is not definitely understood: according to some investigators, the mucus alone is added, and it has no other function than that of annointing the surfaces of the biliary channels and intestines. Rost [39] was convinced that the gallbladder bile, which has 10 times more active biliary alkalies and 8 times more solid substances than that of the liver, contains material of greater importance to the digestive tract than the hepatic duct bile. Furthermore, it has been repeatedly noted in rabbits that the contents of a diseased carrier gallbladder are limpid, free from mucus and correspond in every respect with the secretion obtained from the hepatic duct. In such cases the mucous membrane of the gallbladder is practically destroyed by the subacute inflammation provoked by the vegetating B. typhosus.

The excellent studies of Rous and his collaborators definitely indicate that the gallbladder mucous membrane is endowed with absorptive properties, which act with great rapidity. Observations on cholesterol-

[42] Am. J. Med. Sc., 1917, 153, p. 469.
[43] Lehrbuch der physiologischen Chemie, 1914, p. 390.

ized rabbits by Dewey [44] and ourselves suggest that cholesterol is deposited on the epithelium of the gallbladder as a result of resorption from the bile and is not a product of secretory activity of these cells. In a few experiments primarily conducted for an entirely different purpose, we found that rabbits eliminated a large percentage of the injected cholesterol through the bile. Particularly in the rabbits, which showed anisotropic fat deposits in the gallbladder, the hepatic duct bile contained a rather high percentage of cholesterol. Experiments on cholesterolized rabbits with common duct fistulas therefore supplied valuable information concerning the mooted origin of the biliary cholesterol.

Various other aspects of the function of the gallbladder, as a reservoir for pathogenic micro-organisms thriving either in its contents or its wall, will be presented in the course of our findings on experimental gallbladder carriers.

SUMMARY

The hepatic duct bile of rabbits is always alkaline to litmus and frequently also the phenolphthalein, the P_H^+ varies between 7.4 to 7.7, if examined immediately on withdrawal from the body. The H-ion concentration of this bile decreases steadily on exposure to air on standing and may reach a final P_H^+ of 9.2. If the bile is collected under paraffin oil or put in tightly corked tubes, this change does not take place as readily. The decrease is probably the result of an escape of CO_2 and the absorption of ammonia. The reaction of the bile from the gallbladder is variable; it may be acid, neutral or alkaline, but it is always acid to phenolphthalein. The H-ion concentration of the cystic bile is influenced by the health and the diet of the individual animal and may have a P_H^+ from 6.4 to 7.7, average P_H^+ 7.22. On standing also a decrease in the H-ion concentration takes place which is more rapid for animals kept on base-forming diets. Fasting and acid forming diets produce cystic biles of a high H-ion concentration. Feeding of HCl or injection of $NaHCO_3$ may influence the reaction of the gallbladder bile. Alkaline hepatic duct bile, when neutralized with acid and exposed to the air, regains on standing its original low H-ion concentration.

The hepatic duct and gallbladder biles of guinea-pigs differ little in reaction. The bile is strongly alkaline to litmus and moderately so to phenolphthalein. Fresh bile has a P_H^+ of about 7.5 which changes rapidly, on standing, to a low H-ion concentration.

[44] Arch. Int. Med., 1916, 17, p. 757.

The reaction of the bile from the hepatic duct and from the gallbladder is different in the dog, cat, goat and monkey. The gallbladder bile reaction is always more variable and inclines toward the acid side. The cystic bile of oxen, sheep, and pigs is faintly alkaline to litmus, the P_H^+ ranges between 7.0 and 7.5 on fresh specimens but on heating or exposure to the air it changes steadily in some instances to a P_H^+ above 8.4.

Human fistula bile of one case was faintly alkaline to litmus and faintly acid to phenolphthalein. The P_H^+ 8.0 increased on standing to P_H^+ 8.6. Gallbladder bile from cholecystectomy cases showed a P_H^+ range from 7.7 to 8.6.

Biles derived from typhoid or paratyphoid or streptococcic infected gallbladders of rabbits are alkaline to litmus, and about 50% of them are also alkaline to phenolphthalein. The P_H^+ varies between 7.3 and 7.6 and frequently decreases rather rapidly on standing. They behave in general like hepatic duct bile specimens. Purulent gallbladder specimens may show, on standing, a stationary or even an increasing H-ion concentration, probably due to the formation of lactic acid provoked by the disintegration of cellular material.

The average hourly rate of the bile flow of rabbits is approximately 10 c c and in 24 hours about $\frac{1}{8}$ to $\frac{1}{10}$ of the body weight. One kg. of rabbit secretes 3.76 gm. and 1 kg. of guinea-pig 6.42 gm. of bile per hour. Intravenous injections of sodium taurocholate produce a temporary cholagogue effect in rabbits, dogs and cats. Particularly in the latter species, the familiar cholagogue effect of sodium taurocholate is of great assistance in overcoming reflex inhibition, which follows operative procedures employed in the production of temporary common duct fistulas.

The mechanical and chemical function of the gallbladder is discussed.